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Serum level of cytokeratin 19 as a diagnostic and prognostic marker in patients with HCV-related hepatocellular carcinoma

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Abstract

Background: The heterogeneous nature of human hepatocellular carcinoma (HCC) impedes both treatment strategies and prognostic predictions. Several markers have been proposed for the diagnosis of HCC. Cytoskeleton-associated proteins have been known as cellular integrators in neoplasm formation. Hepatic progenitor cells are thought to express alpha-fetoprotein (AFP) and hematopoietic as well as biliary markers such as cytokeratin 19 (CK 19) and cytokeratin 7. The aim of this study was to verify the role of serum CK 19 alone or in combination with AFP as a diagnostic marker of HCC and to assess the changes in its levels after ablation of HCV-related HCC to evaluate its role as a predictor marker for recurrence of HCC after ablation. The study was conducted on 102 HCV-related cirrhotic patients categorized into three different groups according to the clinical, laboratory, and radiological evaluation: group I—62 patients with early or intermediate HCC who underwent locoregional intervention, group II—20 patients with advanced HCC not fit for any intervention apart from best supportive treatment, and group III—20 cirrhotic patients with no evidence of HCC as proved by two imaging techniques.

Results: The mean serum levels of CK 19 were 6.5 ± 5.7 , 10.5 ± 12.5 , and 6.8 ± 2.8 ng/ml in groups I, II, and III, respectively, with no significant difference between groups. Sensitivity, specificity, positive, and negative predictive values of combined AFP and human CK 19 at cutoff levels of 25.5 ng/ml and 6.25 ng/ml were 93.9%, 45%, 87.5%, and 64.3%, respectively. In group I patients, CK 19 levels were comparable in patients with ablated focal lesion and those who did not at baseline; then, it was significantly higher in ablated patients than in patients with residual tumor 1 and 6 months after the intervention.

Conclusions: Combination of both AFP and CK 19 levels could increase the diagnostic accuracy of suspected HCCs. CK 19 levels are good predictors of ablation/recurrence in patients who underwent interventional procedures minimizing the need for follow-up imaging modalities.

Keywords: Cytokeratin 19, Hepatocellular carcinoma, Ablation, Prognostic

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Background

The heterogeneous nature of human hepatocellular carcinoma (HCC), which represents a serious health problem being the fifth most common malignancy worldwide, and a common cause of death in patients with chronic liver disease [1], impedes both treatment strategies and prognostic predictions [2].

HCC emerges through a process of multistep carcinogenesis [3], and its formation is a subject of discussion and controversy. In fact, other than the different etiological factors, an important cause of its heterogeneity may be the cell type of origin. Formerly, it was suggested that preneoplastic lesions arise from mature hepatocytes subjected to neoplastic transformation [4]. Lately, it has also been proposed that a subset of HCC originates from hepatic progenitor cells (HPCs) [5]. This subset of HCC results enriched for genes expressed earlier in fetal hepatoblasts, including some progenitor cell markers. A continuous upregulation of HPCs was also shown in hepatocellular adenoma [6] and dysplastic nodules [7]. HPC activation was demonstrated to be the most relevant liver carcinogenic condition in chronic viral hepatitis, alcoholic, and non-alcoholic fatty liver disease [8].

Several biliary markers have been proposed for HPCs in HCC including cytokeratin 19 (CK 19) and cytokeratin 7 (CK 7). These cells also express alpha-fetoprotein (AFP) and hematopoietic markers (c-kit, CD34) [9–11].

Cytokeratins are the major filament proteins in the liver where any hepatocyte membrane integrity damage causes their release into the circulation [12]. Moreover, CKs have been known as cellular integrators in several neoplastic changes. Characteristic combinations of CKs are expressed by different epithelia according to the organ of origin and differentiation [13]. An experimental model showed that in vitro primary human CK19-positive tumor cells showed increased invasiveness and that CK-19 knockdown significantly reduced HCC invasive ability [14].

The aim of this study was to verify the role of serum CK 19 alone or in combination with AFP as a diagnostic marker of HCC, to assess the changes in its levels after ablation of HCV-related HCC, and to evaluate the role of CK 19 as a predictor marker for recurrence of HCC after ablation.

Methods

This prospective study was conducted on HCV-induced liver cirrhosis patients with and without HCC according to their clinical, laboratory, and radiological evaluation. They were recruited from the HCC unit at National Hepatology and Tropical Medicine Research Institute (NHTMRI) and the Endemic Medicine Department, Faculty of Medicine, Cairo University.

Study participants

The included groups were as follows: *group I*: 62 cirrhotic patients, 41 males and 21 females, with a mean age (\pm SD) of 56.6 (\pm 5.6) years with early HCC defined as single or three nodules each less than 3-cm diameter with Child-Pugh score A or B. These patients underwent one of the locoregional interventions. *Group II*: 20 cirrhotic patients, 17 males and 3 females, with a mean age (\pm SD) of 55.9 (\pm 5.9) years, with advanced HCC more than 5 cm, they were not fit for any intervention apart from best supportive treatment according to BCLC classification. *Group III*: 20 cirrhotic patients, 9 males and 11 females, with a mean age (\pm SD) of 48.1 (\pm 4.2) years with no evidence of HCC as proved by two imaging techniques (abdominal ultrasound and triphasic CT with contrast) serving as a control group.

Inclusion criteria were (1) males and females above the age of 18 years; (2) post-HCV chronic liver disease, with positive anti-HCV by third-generation enzyme-linked immunosorbent assay (ELISA), detectable serum HCV-RNA by RT PCR and abdominal ultrasonography; and (3) patients in groups I and II with HCC as proved by triphasic CT \pm AFP. Patients with focal lesions other than HCC, history, or evidence of other malignancies; those suffering from any organ failure other than the liver, spontaneous bacterial peritonitis, and hepato-renal syndrome; and previously treated HCC patients were excluded from the study. The protocol was approved by the ethical committee of NHTMRI. After giving an informed consent, the selected patients were subjected to clinical biochemical and imaging assessments; AFP levels were tested by ELISA.

Interventions for patients in group I

Interventional procedure aiming for HCC cure based on BCLC staging system and treatment strategy was performed, for patients with stage A HCC: 45 patients underwent radiofrequency ablation (RFA), and 17 patients underwent microwave ablation (MWA) as the tumor had a close relation to a vessel. Follow-up for tumor recurrence and levels of AFP and CK 19 was carried 1 and 6 months after interventions.

Clinical chemistry analysis

Blood samples were collected and investigated for serum total and direct bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), and albumin on the automated BECKMAN COULTER AU680 autoanalyzer (Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821, USA).

Quantitative detection of CK 19

Serum CK 19 levels were assayed in all patients using the double antibody sandwich ELISA technique supplied

by Sun Red Biotechnology (Shanghai, China). Patients who did not undergo any therapeutic modality were tested only one time at the beginning of this study. Patients who underwent therapeutic interventions were tested for CK 19 at baseline, after 1 month, and 6 months in the follow-up.

Statistical analysis

Data was analyzed using IBM SPSS advanced statistics version 25 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. The chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using the Mann-Whitney test. Comparison between 3 groups was done using the Kruskal-Wallis test; then, post hoc "Scheffe test" was used for pair-wise comparison based on Kruskal-Wallis distribution. Friedman's test was used to compare 3 consecutive measures of numeric variables followed by the Wilcoxon-signed ranks test used to compare two consecutive measures of numerical variables. The receiver operating characteristic (ROC) curve was used for the prediction of cutoff values of markers. All tests were two-tailed. A p value < 0.05 was considered significant.

Results

The comparison of demographic data showed that there was a statistically significant difference between HCC groups and control group in regard to age; the control group was significantly younger than the other 2 HCC groups (p -value < 0.001). Also, the percent of male in group II was significantly higher than controls (p -value 0.024).

Clinical and abdominal ultrasonography data showed that jaundice, pallor, ascites, and lower limb edema were present in 25%, 30%, 50%, and 30%, respectively, of the HCC group not fit for intervention but not present in other groups.

The liver biochemical profile of the studied groups showed that there was a significant difference between group II and the other two groups regarding the mean value of albumin and total bilirubin. Albumin in this group was significantly lower than the other two groups ($p < 0.001$) while total bilirubin was significantly higher. On the other hand, there was no significant difference between all groups regarding the mean value of ALT, AST, and platelet count.

Mean AFP levels were 190.6 ± 852 , 951.9 ± 2323.9 , and 29.3 ± 44.3 ng/ml in groups I, II, and III, respectively, with a significant difference between groups (p value < 0.001). Regarding the CK 19 levels, there was no

significant difference between all groups before any intervention (Table 1).

Using the receiver operating characteristic (ROC) curve, to determine the specificity and sensitivity of human CK 19/AFP alone or combined, the cutoff value of 6.25 ng/ml and 25.5 ng/ml, respectively, were found to be the most suitable (best cutoff) (Table 2).

Studying the characteristics of the focal lesions (FLs), group I showed solitary FL in 90.3% of patients, and size was less than 5 cm in 100% of this group and mainly seated in the right lobe in 82.3%. On the other hand, the FLs were mainly multiple in 70% of group II, and the size of lesions in this group was less than 5 cm in 70% and bi-lobar in 70% of cases.

To assess the prognostic value of CK 19 in HCC patients, the outcome of interventions in group I is displayed in Fig. 1 and the relation of these outcomes to changes in CK 19 levels is shown in Table 3. The CK 19 level was comparable in ablated and residual FL patients at baseline and then was significantly higher in ablated patients than in residual tumor 1 and 6 months after the intervention. Follow-up at 1 month of the intervention, CK 19 median level was 2.9 ng/ml in patients who had no ablation for their FLs compared to it was 15 ng/ml in patients who had ablation. In the follow-up after 6 months, the CK 19 median level was 5.5 ng/ml in patients who had recurrence compared to 17 ng/ml in patients who showed ablation for their focal lesions (p value < 0.001).

Discussion

Early diagnosis of HCC heavily affects the clinical outcome of patients. The widely accepted serological marker for HCC diagnosis is AFP. However, its diagnostic accuracy is controversial and unsatisfactory because of its low sensitivity. Therefore, there is an increasing demand for the discovery of new HCC-specific biomarkers [15]. Moreover, HCCs expressing the biliary/HPC marker CK 19 have been linked with a poor prognosis. Therefore, in this study, we addressed the question of the possibility of using CK 19 as a diagnostic and prognostic marker in HCC [16].

It is established that the incidence of HCC increases progressively with advancing age and it is more

Table 1 Levels of CK 19 in the studied patients

CK 19 levels (ng/ml)	Group I N = 62	Group II N = 20	Group III N = 20	P value
Mean \pm SD	6.5 \pm 5.7	10.5 \pm 12.5	6.8 \pm 2.8	0.054
Median	3.2	5.4	7.2	
Minimum	1.6	2	3	
Maximum	20	45	11.4	

CK 19 cytokeratin 19

Table 2 Sensitivity, specificity, positive, and negative predictive values of AFP and CK 19 in studied patients

	Best cutoff	SN	SP	PPV	NPV
AFP	25.5 ng/ml	80.5%	75%	93%	48.4%
CK 19	6.25 ng/ml	63.4%	55%	85.2%	26.8%
Combined AFP and CK 19	25.5 ng/ml and 6.25 ng/ml	93.9%	45%	87.5%	64.3%

AFP alpha-fetoprotein, CK 19 cytokeratin 19, SN sensitivity, SP specificity, PPV positive predictive value, NPV negative predictive value

predominant in males [17]. This comes in agreement with the demographic data of the studied patients. In this study, we found a statistically significant difference between the HCC group not fit for intervention (group II) and the other two groups regarding the mean value of albumin and total bilirubin, this could be explained by the fact that lower albumin and higher bilirubin levels are indicators of a higher Child-Pugh score hindering the possibility of interventional therapy.

In the current study, the AFP levels were significantly elevated in the HCC groups compared to the cirrhosis group. This is in agreement with several previous studies [18, 19]. However, this was not universally agreed [20]. The cutoff value for diagnosis, sensitivity, and specificity showed wide variability among different studies. Our results showed the sensitivity and specificity of serum AFP were 80.5% and 75%, respectively, with a cutoff value of 25.5 ng/ml for HCC diagnosis. A study demonstrated that the sensitivity and specificity of AFP at a cutoff value of 10.86 ng/ml were 75% and 92%, respectively [21]. Spangenberg et al. reported that at a cutoff value of 20 ng/ml, serum AFP showed 60–80% sensitivity, although this sensitivity decreases to about 40% for the

detection of small tumors [22]. The use of a higher cutoff value such as 200 ng/ml drops the sensitivity to 22% while increases the specificity [23]. Therefore, the use of AFP in clinical practice is limited by the low sensitivity at cutoff values maintaining sufficiently high specificity. These discrepant results warranted exploring the usage of additional biomarkers to improve the diagnostic accuracy.

CK 19 is an HCC-cancer stem cell marker that plays an integral role in carcinogenesis, metastases, and recurrence [24]. In our study, although there was no significant difference in baseline serum CK 19 levels among the three groups, its combination with AFP improved their sensitivity to 93.9%.

To verify the prognostic role of CK 19, we demonstrated that baseline CK 19 levels in group I were significantly lower than 1- and 6-month levels post-intervention in those who showed ablation of FLs.

A large meta-analysis was done by *Da-wei Sun and his colleagues in 2015* to investigate the association between CK 19 expression in tissue and the prognosis of HCC patients. It included 17 studies with a total of 2943 patients. The results showed that tissue CK 19

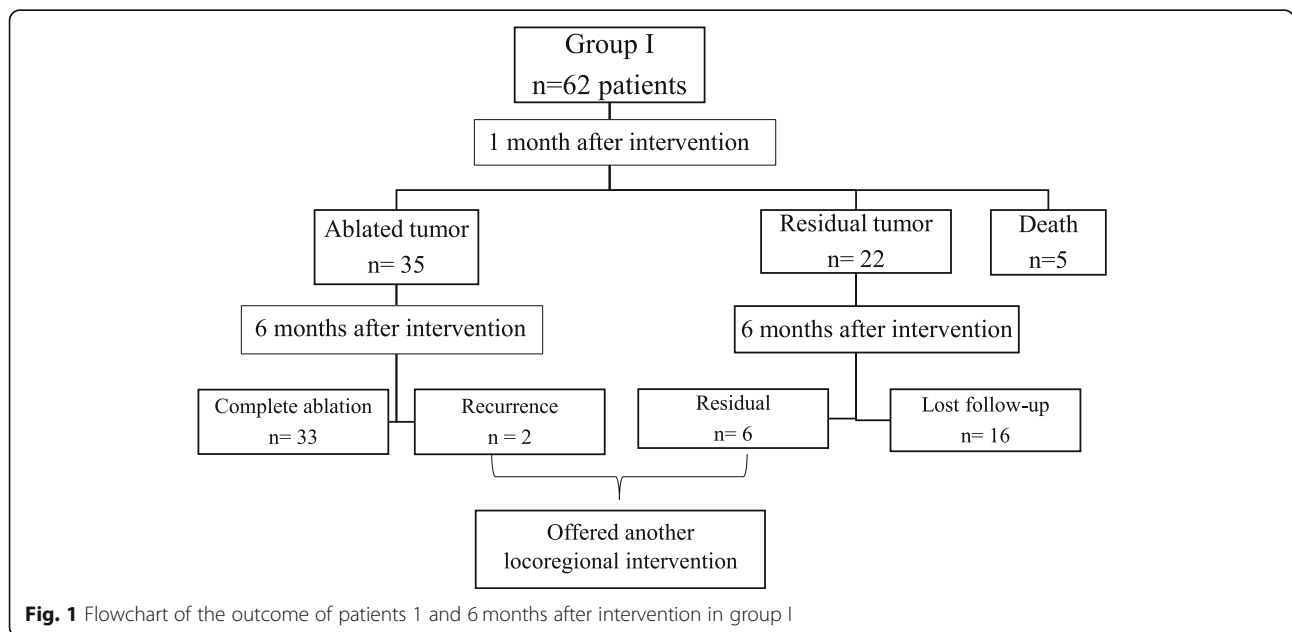


Fig. 1 Flowchart of the outcome of patients 1 and 6 months after intervention in group I

Table 3 Relationship between CK 19 levels and the status of the focal lesions according to the final outcome after 6 months

	Focal lesions by CT						p value
	Residual tumor (n = 8)			Ablated (n = 33)			
	Median	Minimum	Maximum	Median	Minimum	Maximum	
CK 19 before intervention	3.4	1.6	16.0	3.0	1.7	20.0	0.706
CK 19 1 month after intervention	2.9	2.0	10.0	15.0	7.8	46.0	< 0.001*
CK 19 6 months after intervention	5.5	3.0	9.0	17.0	4.0	40.0	< 0.001*
p value	0.223			< 0.001*			

*p value is significant. CK 19 cytokeratin 19

overexpression was significantly associated with poor overall survival rate and early tumor recurrence rate in HCC patients [25].

This difference in results may be attributed to many factors: one of them is the number of candidates that was smaller in our study, and another factor is the method of detection of CK 19 which was by measuring its serum level by ELISA in our study but in other studies was immunohistochemistry of surgically excised tumors or using microarrays. Another factor is a longer period of follow-up in other studies, and this may reveal cases of recurrence or new lesions on long follow-up period.

In our study, we found elevation of serum level of CK 19 after ablation of tumor by single or combined locoregional intervention modalities. These interventions led to damage of tumor cells by necrosis and apoptosis which in turn may have led to the release of CK 19 in serum and this may explain partially our results especially that patients with residual tumors did not show a significant increase of CK 19 levels. This explanation is supported by a study performed by Vanagas and his colleagues who detected apoptosis in the transition zone at a distance of 9–10 mm from the rim of the necrotic zone 1 h after local ablation procedure [26]. Another theory for the increased levels of CK 19 as a stemness marker is the occurrence of hypoxia post-intervention [27]. However, more studies are needed to see the effect of liver resection on the level of CK 19 before and after resection.

Limitations of this study include the short duration of and loss of some patients during the follow-up period. The strength of this study is the use of serum CK 19 levels measured by simple ELISA technique instead of the repeatedly reported tissue expression method.

Conclusions

Measurement of both CK 19 and AFP combined could be a simple, noninvasive way to increase the diagnostic accuracy of suspected HCCs and evaluate treatment response and prognosis in patients with HCC amenable for intervention. Post-ablation elevation of serum CK 19 levels can predict complete HCC ablation before doing imaging modalities.

Abbreviations

AFP: Alpha-fetoprotein; CK 19: Cytokeratin 19; FL: Focal lesion; HCC: Hepatocellular carcinoma; HPC: Hepatic progenitor cell; MWA: Microwave ablation; RFA: Radiofrequency ablation; ROC: Receiver operating characteristic

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Not applicable.

Authors' contributions

MER, HA, and AE were responsible for the concept, plan of the work, interpretation of results, and final revision. TAM was responsible for patients' inclusion, intervention performance, and follow-up. RMM was responsible for writing the manuscript. SME and SAS were responsible for laboratory work. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted after approval of the ethical committee of the National Hepatology and Tropical Medicine Research Institute. No available reference number. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Written informed consent was obtained from all participants included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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